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PATENT
Attorney Docket No: HYS-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Boyle, *et al.*
Serial No: 09/545,283
Filed: April 7, 2000
For: METHODS AND MATERIALS
RELATING TO NOVEL C-TYPE
LECTIN RECEPTOR-LIKE
POLYPEPTIDES AND
POLYNUCLEOTIDES
Examiner: Jehanne E. Souaya
Group: 1634

HAND DELIVERY

DECLARATION OF WALTER FUNK, Ph.D., UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, DC 20231

Sir:

I, Walter Funk, Ph.D., hereby declare as follows that:

1. I received a doctoral degree in Biochemistry from the University of British Columbia. Since receiving my Ph.D., I have been employed by the Geron Corporation and Hyseq, Inc. d/b/a Hyseq Pharmaceuticals ("Hyseq") as a scientist and manager of Molecular Biology and Genomics. I am currently the Vice President of Research at Hyseq.

2. I have reviewed the specification of the above-identified application and make statements in this declaration to address a rejection regarding utility made in the Office Action mailed February 27, 2002 wherein the Examiner alleges that the specification does not teach the activity or biological function of the C-type lectin receptor-like polypeptide of SEQ ID NO:4.

3. The C-type lectin receptor-like polypeptide of SEQ ID NO:4 exhibits homology with mouse macrophage C-type lectin receptor, human dendritic cell immunoreceptor (DCIR), human C-type lectin receptor DDB27, and mouse C-type lectin receptor as shown in Figures 1 to 4 of the specification and Exhibit 1 hereto. The polypeptide of SEQ ID NO:4 is 39% identical to mouse macrophage C-type lectin, 49% identical to human dendritic cell immunoreceptor DCIR, 49% identical to human C-type lectin receptor DDB27, and 44% identical to mouse C-type lectin receptor. Further, the polypeptide of SEQ ID NO:4 exhibits 99% identity the blood dendritic cell antigen 2 (BDCA-2), a plasmacytoid dendritic cell-specific type II C-type lectin (Dzionic, *et al.*, *J. Exp. Med.* 194(12):1823-1834 (2001)); International Patent Application No. WO 01/36487 A2) (See Exhibit 2) . This homology to the known C-type lectin receptor family members strongly suggests that C-type lectin receptor-like polypeptide of the present invention is a novel member of the C-type lectin receptor family and would possess similar biological activity as the known C-type lectin family members.

4. An alignment of SEQ ID NO:4 with the amino acid sequences of other C-type lectin receptor proteins, including blood dendritic cell antigen 2 (BDCA2)/dendritic cell lectin (DLEC), DCIR, and C-type lectin 6 (CLEC-6), is shown in Exhibit 2. The C-lectin domain extends from amino acid 100 to amino acid 206 of SEQ ID NO:4 as determined by Pfam analysis (Sonnhammer, *et al.*, *Nucleic Acids Res.* 26:320-322 (1998)), and the C-type consensus sequence extends from amino acid 180 to 206, as determined by PROSITE analysis (Falquet, *et al.*, *Nucl. Acid Res.* 30:235-238 (2002)). A mannose or glucose binding specificity is indicated from the amino acid motif, glutamic acid/proline/asparagine (EPN) starting from amino acid 172 to 174 of SEQ ID NO:4 and DLEC/BDCA-2, similar to mouse macrophage receptor. By comparison, DCIR has the variant amino acid motif, glutamic acid/proline/serine (EPS) which confers binding specificity for galactose, whereas hepatic asialoglycoprotein receptors-1 and -2 (ASGPR-1 and -2) have the amino acid motif, glutamine/proline/aspartic acid (QPD) that binds N-acetylgalactosamine. Type II members of the C-type lectin receptor proteins are characterized by a single carbohydrate recognition site (CRD) which has one calcium-binding domain per CRD, wherein the domain consists of calcium binding residues at amino acid 146, amino acids 172-174, 178-179 and amino acids 194-195. The cysteines that participate in the disulphide bond formation and can potentially aid in dimerization are also conserved, four within the CRD domain at residues 111, 180, 198, and 206. There are two additional cysteines present at amino acids 82 and 94 that may

be optionally involved in disulphide linkage. Thus, the polypeptide of SEQ ID NO:4 shares in common the C-lectin domain, C-type consensus sequence, amino acid motifs specific for mannose or glucose binding and CRD with requisite calcium-binding domains and N-glycosylation sites with other members of the C-type lectin protein family, particularly BDCA-2.

5. Using eMATRIX software package (Stanford University, Stanford CA) (Wu, et al., J. Comp. Biol. 6:219-235 (1999)) at a threshold e-value of 1e-08, the C-type lectin receptor-like polypeptide of SEQ ID NO: 172 was determined to have the following twenty-four (2) eMATRIX C-type lectin domain hits. The results describe: e-value, score, Accession number, domain name, and range of amino acid residues of SEQ ID NO:172 that correspond to the eMATRIX domain:

-e value	Score	Accession No.	Domain Name	Amino Acid Range
2.731e-09	12.25	BL00615B	C-type lectin domain	193-206
9.400e-09	16.68	BL00615A	C-type lectin domain	94-111

Using the Pfam software program (Sonnhammer, *et al.*, *Nucleic Acids Res.* 26:320-322 (1998)), C-type lectin receptor-like protein of SEQ ID NO:4 is predicted to contain one (1) lectin C-type domain wherein the score is 97.7, E-value 2.3e-25, and amino acid sequence encoded (start and end amino acid position) is:

GMQSWTKSQKNCSVMGADLVVINTTEEHDFIIHNL

KRNSSYFLGLSHPRGRRHWQVDHTPYNENVTFWHSGEPPNNLDERCAIINFRSSQE
WGWNDIHCHVPHKSICEM (100-208). Thus the eMATRIX and Pfam results describe the presence of C-type lectin domains proteins consistent with SEQ ID NO:4 having C-type lectin receptor-like activity and being a member of the C-type lectin receptor family.

6. mRNA expression studies demonstrated in Table 1 (Exhibit 3) that SEQ ID NO:4 mRNA was expressed almost exclusively in immune cells and tissues. The expression of SEQ ID NO:4 was observed in resting CD4+ cells and CD19+ cells, but not in activated CD4+ cells and activated CD19+ cells. The findings that C-lectin receptor-type

expression is detected in resting B-cells and T-cells but not activated B-cells and T-cells suggest a role for this protein in lymphocyte activation and/or differentiation similar to that of another type II C-type lectin receptor, CD23 (Mossalyi *et al. Blood.* (1990) 75:1924-1927; Ouaz *et al. Blood.* (1994) 84(9):3095-3104; Fournier *et al. Blood.* (1994) 84(6):1881-1886). In addition, expression of SEQ ID NO:4 mRNA was detected in peripheral blood mononuclear cells, tonsil, spleen, peripheral blood leukocytes, fetal spleen, placenta, lung and testis. As described in the specification, at page 43, lines 6-10, C-type lectin receptors are involved in inflammatory diseases, such as asthma. For example, C-type lectin receptor may play a role in modulating the inflammatory response associated with allergic airway disease by phagocytosis and antigen uptake (Currie, *et al., J. Immunol.* 164(7):3878-86 (2000)). CD23 in addition to being involved in lymphocyte differentiation is also implicated in inflammation (Hazku *et al. Am J. Respir. Crit. Care Med.* 161:952-960 (2000); Kleinau *et al. J. Immunol.* 162(7):4266-4270 (1999)). These findings that C-lectin receptor-type expression is detected in immune cells and tissues are consistent with a role of C-type lectin receptor-like polypeptides in immune-related reactions, such as inflammation and asthma.

7. The Examiner has noted on page 9 of the Office Action that "the specification asserts that SEQ ID NO:4 may function as a shed receptor, however the specification has not demonstrated such..." Applicants have conducted Western Blot studies to demonstrate that the polypeptide encoded by SEQ ID NO:4 is indeed secreted or cleaved from the cell surface. As shown in Exhibit 4, the protein encoded by SEQ ID NO:4 was cloned into a vector to generate a construct with a tag to detect secretion. The construct was transfected into human embryonic kidney (HEK293) cells and after two days, the cells and supernatant screened for the tagged protein. The presence of SEQ ID NO:4 on cell surface and in the cell culture medium was analyzed by performing western blot analysis using a mouse anti-V5 primary antibody and HRP-conjugated goat anti-mouse secondary antibody. Anti-V5 staining was detected by chemiluminescence. The detection of receptor protein in the supernatant when expressed on HEK293 cells suggests that the receptor does not remain on the cell surface even though there is a definite predicted transmembrane domain present from amino acid 24-36. As shown in Exhibit 4, the protein was predominantly detected in the supernatant, indicating that SEQ ID NO:4 is secreted or more likely cleaved from the cell surface in these cells. Thus, laboratory data provides confirmation that the polypeptide of SEQ ID NO:4 is a shed receptor.

8. Another member of the C-type lectins is L-selectin, a calcium-dependent C-type lectin known to mediate the rolling and tethering of leukocytes on endothelial surfaces, which is a prerequisite for leukocyte adhesion and extravasation. In particular L-selectin mediates homing of naïve lymphocytes via endothelial veins to peripheral lymph nodes and Peyer's patches and also plays a role in recruitment of leukocytes to inflammatory sites. *In vitro*, association of L-selectin with GlyCAM-1 can activate beta2 integrins. L-selectin is expressed by most hematopoietic cells at some stage of differentiation and its localization at the tips of the microvilli is required for optimal adhesion. Interestingly, L-selectin was shown to be shed as a result of activation of leukocytes (Tedder, *et al.*, *FASEB J.* 9, 866-873 (1995)). CD23, another type II C-type lectin, is also known to be shed and the soluble portion has immune activity (Mossalayi *et al. Blood.* (1990) 75:1924-1927). Similarly, as shown in Exhibit 4 and described above, SEQ ID NO:4 was shown to be secreted or cleaved from the cell surface much like CD23 and L-selectin that are also known to be shed (Mossalayi *et al. Blood.* (1990) 75:1924-1927; Ouaz *et al. Blood.* (1994) 84(9):3095-3104; Fournier *et al. Blood.* (1994) 84(6):1881-1886, Tedder *et al. FASEB J.* 9.866-873 (1995)). Similar to L-selectin and CD23, the function of SEQ ID NO:4 could be regulated by shedding from immune cells and the shed protein may itself play additional role in modulating immune response.

9. The Specification teaches that the C-type lectin receptor-like polypeptide of SEQ ID NO:4 is homologous to plasmacytoid dendritic cell-specific antigen-2 (BDCA-2), a Type II C-type lectin, known to mediate antigen capture and to inhibit interferon α/β induction (Dzionek, *et al.*, *J. Exp. Med.* 194(12):1823-1834 (2001)). Analysis of BDCA-2 mRNA expression by PCR showed that BDCA-2 is selectively expressed in plasmacytoid dendritic cells (PDCs). Similar to other C-type lectin receptors, BDCA-2 also was shown to exhibit antigen uptake as demonstrated by studies using Ag-mAb complexes, and induced a rapid and transient rise in intracellular calcium, consistent with a role in antigen capture and a calcium-mediated signal transduction pathway. Blocking of BDCA-2 with monoclonal antibodies inhibited the induction of IFN α/β induction by viruses and inflammatory factors. The activity of C-type lectin receptors, including BDCA-2, and homologous proteins, such as SEQ ID NO:4, to mediate antigen capture and modulate inflammatory mediators, specifically IFN α/β , is a specific utility, inasmuch as it is not a utility shared by all polypeptides. Because of the ability of C-type lectin proteins to regulate immune activity, C-type lectin receptor-like polypeptides, such as SEQ ID NO:4, may be

useful in the management and/or treatment of immune-related disorders. For example, systemic lupus erythematosus (SLE), an inflammatory disease, is characterized by increased levels of IFN α/β , which play a role in the pathogenic mechanism of SLE. The administration of anti-BDCA-2 mAb to SLE patients provides another therapy for inhibiting IFN α/β by PDCs. The Specification teaches the use of these C-type lectin receptor polypeptides for the management and/or treatment of inflammatory disorders (See Specification, 5.8.7 IMMUNE STIMULATING OR SUPPRESSING ACTIVITY, page 55, line 6 to page 59, line 17; 5.8.15 ANTI-INFLAMMATORY ACTIVITY, page 68, line 12 to page 69, line 4; 5.8.18 ARTHRITIS AND INFLAMMATION, page 71, line 26 to page 72, line 11)

10. All of the evidence discussed in paragraphs 3-9 support the conclusion that C-type lectin receptor-like polypeptide is a novel member of the C-type lectin receptor family and one skilled in the art would expect C-type lectin receptor-like polypeptide to exhibit C-lectin receptor type activity. Restricted expression in immune cells suggests a role of this lectin in the immune response including inflammation and other immune-related disorders. In addition, the observation that this receptor can be shed from the cell surface suggest that shedding may regulate its activity similar to what has been shown for other C-type lectins such as CD23 and L-selectin. The C-type lectin receptor-like polypeptide of SEQ ID NO:4, therefore has a specific and substantial utility as a mediator of these immune processes involved in inflammation.

11 I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: JUL 25, 2002


Walter Funk, Ph.D.

BLASTP ALIGNMENT OF C-TYPE LECTIN RECEPTOR-LIKE WITH MOUSE MACROPHAGE C-TYPE LECTIN

Score = 412 (145.0 bits), Expect = 1.2e-37, P = 1.2e-37

Identities = 85/215 (39%), Positives = 119/215 (55%), Frame = +1

SEQ ID NO. 4 55 EEPDREKGLWMFOLKWSMAVVISLLSVCFVSSVVPENFMYSKTVKRLSKLREYQY 234
C-Type Lectin Receptor-Like: EE Q + KG QL AVSI LS CF + +V H++ T + KL +Y

Macrophage C-Type Lectin: 4 EESQMSKSGTRHPQLIPCFEAVVISISFLSACFISTCLVTHHYFLRMTRGSVVKLSDY--- 60

SEQ ID NO. 4 235 HSSLTCVME---GKDIEDWSCPTPWTSEQSSCYFISTGMQSWTKSQKNCSVMGADLV 402
C-Type Lectin Receptor-Like: H+ +TC+ E G W+CCP W +FQS+CYF Q+W +S++NCS M + LV

Macrophage C-Type Lectin: 61 HTRVTCIREFPQPGATGTTCCPVSWRAFQSNCFPLNDNOTWHESEKNCSGMSHLVT 120

SEQ ID NO. 4 403 INTTEHDEFIHNLRNSSYFLGLSHPRGRHWQWVDHTPYNENVTFWHSGEPNN-LDER 579
C-Type Lectin Receptor-Like: INT E +F+ L + SYFLGL+ WQWVD TP+N + FW GE N+ ++E

Macrophage C-Type Lectin: 121 INTEAEQNEFTQLIDKRFSYFLGLADENVEGQWQWVDKTPENPHTVFEKEGESNDFMEED 180

SEQ ID NO. 4 580 CAIINFRSSQEWGWNNDIHCHVPHKSICEMKKIYIMKYS 696
C-Type Lectin Receptor-Like: C ++ ++W WND CH + IC++ I K S

Macrophage C-Type Lectin: 181 CVVL-VHVHEKVVWVNDPCHFEVRRICKLPGITFNWKPS 218

FIG. 1

BLASTP ALIGNMENT OF C-TYPE LECTIN RECEPTOR-LIKE WITH DENDRITIC CELL IMMUNORECEPTOR

Score = 529 (186.2 bits), Expect = 4.8e-50, P = 4.8e-50

Identities = 93/188 (49%), Positives = 130/188 (69%), Frame = +1

SEQ ID NO. 4 118 VSIILLVCFVSSVPHNFMYSKTVKRLSKLREYQOYHSSLTCVMEGKDIED--WSCC 291
C-Type Lectin Receptor-Like: ++ LLL++ F ++ V+ + K + L K + H++L CV + +E+ WSCC

Dendritic Cell Immunoreceptor: 51 LIFFLLAISFFIAFVI-----FFQKYSQLLEKKTTELKELVHTLLECVKKNMPYEETAMSCC 106

SEQ ID NO. 4 292 PTPWTSFQSSCYFISTGMQSWTKSOKNCSVMGADLVVINTTEHDFIHNLRNSSFYFLG 471
C-Type Lectin Receptor-Like: P W SF S+CYFIST SW S+K+C+ M A L+VINT EE DFI NL+ S+YF+G

Dendritic Cell Immunoreceptor: 107 PKNWKSSSNCFISTESASWQDSKDCARMEAHLLVINTQEQDFIFQNLQESAYFVG 166

SEQ ID NO. 4 472 LSHPRGRHHQWVDHTPYNENVTFWHSGEPNNLDERCAIINFRSS-QEWGNDIHCHVPH 648
C-Type Lectin Receptor-Like: LS P G+RHQWVD TPYNE+ TEWH EP++ +ERC ++NFR S + WGMND++C P

Dendritic Cell Immunoreceptor: 167 LSDPEGQRHMQWVDQTPYNESSTFWHPREPSDPNERCVLNFRRKSPKRWGMNDVNCIGPQ 226

SEQ ID NO. 4 649 KSICEMKKIYI 681
C-Type Lectin Receptor-Like: +S+CEM KI++

Dendritic Cell Immunoreceptor: 227 RSVCEMMKIH 237

BLASTP ALIGNMENT OF C-TYPE LECTIN RECEPTOR-LIKE WITH C-TYPE LECTIN DDB27

Score = 529 (186.2 bits), Expect = 4.8e-50, P = 4.8e-50

Identities = 93/188 (49%), Positives = 130/188 (69%), Frame = +1

SEQ ID NO. 4
C-Type Lectin Receptor-Like:

118 VSIILLVCFVSSVPHNFMYSKTVKRLSKLREYQYHSSILTCWEGKDIED--WSCC 291
++ LLL++ F ++ V+ + K + L K + H++L CV + +E+ WSCC
51 LIFELLALISFIAFYI----FFQKYSQLEKKTTELKLVHTTLECVKKNMPVEETAMSCC 106

SEQ ID NO. 4
C-Type Lectin Receptor-Like:

292 PTPWTSFQSSCYFTSTGMQSWTKSQKNCVSGADLVINTTEHDFIHNLRNSSYELG 471
P W SF S+CYFIST SW S+K+C+ M A L+VINT EE DFI NL+ S+YF+G
107 PKNWKSFFSSNCYFTSTESASQDSEKDCARMEALLVINTQEEQDFIQNLQESAYFVG 166

SEQ ID NO. 4
C-Type Lectin Receptor-Like:

472 LSHPRGRHRHWQWDHTPYNENVTFWHSGEPNNLDERCAIINFRSS-QEWGWNDIHCHVP 648
LS P G+RHQWQVD TPYNE+ TFWH EP++ +ERC ++NFR S + WGWND++C P
167 LSDPEGQRHWQWDQTPYNESSTFWHPRFSPDENRCVVLNFRKSPKRWGWNQVNCIGPQ 226

SEQ ID NO. 4
C-Type Lectin Receptor-Like:

649 KSICEMKKIYI 681
+S+CEM KI++
DDB27: 227 RSVCEMMKIHL 237

FIG. 3

BLASTP ALIGNMENT OF C-TYPE (CALCIUM DEPENDENT, CARBOHYDRATE RECOGNITION DOMAIN) LECTIN RECEPTOR-LIKE,
SUPERFAMILY MEMBER 6

Score = 448 (157.7 bits), Expect = 1.8e-41, P = 1.8e-41

Identities = 92/209 (44%), Positives = 130/209 (62%), Frame = +1

SEQ ID NO. 4	49	PEEPP-QD-REKGLWFFQLKWMSMAVVSIILLVSCFTVSSVVPNEMYSKTVKRLSKLRE	222
C-Type Lectin Receptor-Like:		P E+P +D R+ G L + S+ ++ +LL++ F V+ ++ YS+ ++ +	
Mouse C-Type:	29	PREKPIRDLRKPGSP--SLLLTSLML-LLLAITFLVAFIIFYQ-KYSQLEEKRAKN	84
SEQ ID NO. 4	223	YQYHSSLTCVMEKDIED--WSCCPFPWTSFQSSCYFIST--GMQSWTKSQKNCVMGA	390
C-Type Lectin Receptor-Like:		H+ L C +ED WSCP W F S CY + T SW KS++NCS MGA	
Mouse C-Type:	85	IM--HNELNCTKSVPMEKVMWSCPMDRLFGSHCYLVPFTVSSSASMNKSEENC SRMGA	142
SEQ ID NO. 4	391	DLVINTTEHDFLIHNLKRNSYFLGLSHPRGRHWQVDHTPYNENVTFWHSGEPNL	570
C-Type Lectin Receptor-Like:		LVVI + EE DFI L +++YF+GL G R WQVD TPY E++TFWH+GEP++	
Mouse C-Type:	143	HLVVIQSQEEQDFITGLDTHAAYFIGL-WDTGHRQWQVDQTPYEESITFWHNGEPSSG	201
SEQ ID NO. 4	571	DERCAIINERSSQEWGWNIDHCHVPHKSIEMKKI	675
C-Type Lectin Receptor-Like:		+E+CA I +R WGWNDI C + KS+C+MKKI	
Mouse C-Type:	202	NEKATIITYRWKTGMGWNDISCSLKQKSVQCMKKI	236

FIG. 4

BLASTP Alignment Of C-Type (Calcium Dependent, Carbohydrate Recognition Domain) Lectin Receptor-Like Superfamily Member 6

Score = 448 (162.8 bits), Expect = 6.7e-48, P = 6.7e-48
Identities = 92/215 (42%), Positives = 130/215 (60%)

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SEQ ID NO.4 :      3 PEEEP-QD-REKGLWVFQLKVSMVAVSVILLVCFIVSSVVPHPNFMYSKTVKRLSKLRE 60
Mouse C-Type:      29 PREKPIRDLRKPSP--SLLTSLMLL-LLLLAITFLVAFIIFYQ-KYSQLEEKKAAXN 84

SEQ ID NO.4 :      61 YQYHSSLTCVMECKDIED--WSCCPTPWTSEFQSSCYFIST--GMQSWTKSQKNCSVMGA 116
Mouse C-Type:      85 IM--HNEINCTKSVSPMEDKVVSCCPKDWRLFGSHCYLVPTVSSASWNKSEENC SRMGA 142

SEQ ID NO.4 :      117 DLVVINTTEEHDFIINHNLKRNSSYFLGLSHPRGRRHQWVDHTPYNENVTFWHSGEPNNL 176
Mouse C-Type:      143 HLVVIQSQEEQDFITGILDTHAAYFIGL-WDTGHRQWQWVDQTPYEESITFWHNGEPSSG 201

SEQ ID NO.4 :      177 DERCAIINFRSSQEWGWNDIHCHVPHKSIKCEMKKI 211
Mouse C-Type:      202 NEKCATIIRWKTGWGWNDISCSLKQKSVQCMKKI 236

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FIG. 4 (Corrected)

Tuesday, July 23, 2002 5:15 PM

[illegible]

33	S	V	C	F	T	V	S	S	V	V	P	H	N	-	F	M	Y	S	K	T	V	K	R	L	S	K	L	R	E	Y	00002860Fff201_1
33	S	V	C	F	T	V	S	S	V	V	P	H	N	-	F	M	Y	S	K	T	V	K	R	L	S	K	L	R	E	Y	DLEC_BDCA2
11	-	-	-	-	-	-	-	-	V	P	H	N	-	F	M	Y	S	K	T	V	K	R	L	S	K	L	R	E	Y	DLEC_short_isoform	
58	A	I	S	F	F	I	A	F	V	I	-	-	-	-	F	F	Q	K	Y	S	Q	L	L	E	K	K	T	T	K	gi5823974_DCIR	
32	G	V	C	F	I	A	S	C	L	V	T	H	H	N	F	S	R	C	K	R	G	T	G	V	H	K	L	-	-	-	gi17226268_CLEC-6

	W		*	Y		W		*																							
87	W	T	S	F	Q	S	S	C	Y	F	I	S	T	G	M	Q	S	W	T	K	S	Q	K	N	C	S	V	M	G	A	00002860Ff201_1
87	W	T	S	F	Q	S	S	C	Y	F	I	S	T	G	M	Q	S	W	T	K	S	Q	K	N	C	S	V	M	G	A	DLEC_BDCA2
56	W	T	S	F	Q	S	S	C	Y	F	I	S	T	G	M	Q	S	W	T	K	S	Q	K	N	C	S	V	M	G	A	DLEC_short_isoform
110	W	K	S	F	S	N	C	Y	F	I	S	T	E	S	A	S	W	Q	D	S	E	K	D	C	A	R	M	E	A	gi5823974_DCIR	
88	W	R	A	F	O	S	N	C	Y	F	P	L	T	D	N	K	T	W	A	E	S	E	R	N	C	S	G	M	G	A	gi17226268_CLEC-6

	W/F W V D Q/G												W												+						
147	P	R	G	R	R	H	W	Q	W	V	D	H	T	P	Y	N	E	N	V	T	F	W	H	S	G	E	P	N	N	L	00002860Ff201_1
147	P	G	G	R	R	H	W	Q	W	V	D	Q	T	P	Y	N	E	N	V	T	F	W	H	S	G	E	P	N	N	L	DLEC_BDCA2
116	P	G	G	R	R	H	W	Q	W	V	D	Q	T	P	Y	N	E	N	V	T	F	W	H	S	G	E	P	N	N	L	DLEC_short_isoform
170	P	E	G	Q	R	H	W	Q	W	V	D	Q	T	P	Y	N	E	S	S	T	F	W	H	P	R	E	P	S	D	P	gi5823974_DCIR
148	E	N	A	K	G	O	W	R	W	V	D	Q	T	P	F	N	P	R	R	V	F	W	H	K	N	E	P	D	N	S	gi17226268_CLEC-6

W N D S/W

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177 D - E R C A I I N F R - S S Q E W G W N D I H C H V P H K S 00002860Ff201_1
177 D - E R C A I I N F R - S S E E W G W N D I H C H V P Q K S DLEC_BDCA2
146 D - E R C A I I N F R - S S E E W G W N D I H C H V P Q K S DLEC_short_isoform
200 N - E R C V V L N F R K S P K R W G W N D V N C L G P Q R S gi5823974_DCIR
178 Q G E N C V V L V Y - - N Q D K W A W N D V P C N F E A S R gi17226268_CLEC-6
* PS00615

205 I C E M K K I Y I Y M K Y S P W K C V W V G I H R C R K L N 00002860Ff201_1
205 I C K M K K I Y I DLEC_BDCA2
174 I C K M K K I Y I DLEC_short_isoform
229 V C E M M K I H L gi5823974_DCIR
206 I C K I P G T - - - - - T L N gi17226268_CLEC-6
* PS00615

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Decoration 'Decoration #1': Box residues that match 00002860Ff201_aa1 exactly.

KEY

Bold Residues = Lectin/crd PFAM signature (PF00059)

Underlined Residues = PROSITE domain for c-lectin (PS00615)

Markings Above the Alignment (J. Immunol. 1999, 163:1973-1983):

Dots (.)= Calcium-binding residues.

Asterisks (*) = Intramolecular Disulfide Cysteines

+++ = Mannose/glucose recognition motif.

Canonical c-lectin residues are indicated above their respective positions.

EXPRESSION OF SEQ ID NO: 4 IN IMMUNE CELLS

Expression of SEQ ID NO: 4 was determined by PCR in 1st strand cDNA panels of various healthy tissues, including adult prostate, testis, placenta, ovary, pancreas, small intestine, colon, lymph node, tonsil and bone marrow and adult and fetal heart, brain, lung, liver, skeletal muscle, kidney, spleen and thymus. A panel of peripheral blood cells, including total peripheral blood leukocytes, resting and activated mononuclear cells, resting and activated CD4+ cells, resting and activated CD8+ cells, resting CD14+ cells, and resting and activated CD19+ cells, was also used (MTC™ panels, Clontech, Palo Alto, CA). In addition, 1st strand cDNA isolated using standard techniques from immature and mature monocyte derived dendritic cells (method in Wilkin et al., 2001, J Immunol.166(12):7172-7), was analyzed. Glucose 3-phosphate dehydrogenase (G3PDH) mRNA expression was used as a positive control and normalization factor in all samples. PCR was performed using 2 ul of each cDNA template for a total of 35 or 25 cycles for SEQ ID NO: 4 and G3PDH, respectively. The amplification product was detected by analysis on agarose gels stained with ethidium bromide.

The following quantification scale for the PCR expression data was used: “-“ = no detectable expression; “+” = low expression; “++” = intermediate expression, “+++” = strong expression; Expression of SEQ ID NO: 4 in immune cells is indicated in Table 1, expression in non-immune cells in Table 2..

Table 1

Tissue	Relative Fold Increase
Peripheral Blood Mononuclear cells	+++
Activated mononuclear cells	-
Resting CD4+ cells	+
Activated CD4+ cells	-
Resting CD8+ cells	-
Activated CD8+ cells	-
Resting CD19+ cells	++
Activated CD19+ cells	-
Resting CD14+ cells (Monocytes)	-

Resting monocyte derived dendritic cells	-
Activated monocyte derived dendritic cells	-
Tonsil	+
Spleen	+
Lymph Node	-
Thymus	-
Peripheral Blood Leukocytes	++
Bone Marrow	-
Fetal Liver	-
Fetal Spleen	+
Fetal thymus	-

The results shown in Table 2 demonstrate the expression of SEQ ID NO: 4 mRNA in non- immune organs. No expression of SEQ ID NO: 4 was detected in any of the other tissues tested.

Tissue	Relative Fold Increase
Placenta	+
Lung	+
Testis	+

SEQ ID NO: 4 IS SECRETED BY HEK293 CELLS

The full length ORF encoded by SEQ ID NO: 4 was cloned in frame into the mammalian expression vector pCDNA3.1/V5-His-Topo (Invitrogen, Carlsbad, CA), to generate a c-terminally V5/His tagged expression construct. The resulting plasmid was transiently transfected into HEK293 cells using the Fugene 6 transfection reagent (Roche, Indianapolis, IN), according to manufacturers instructions. 48 hours post transfection cells (A) and supernatant (B) were collected and analyzed by westernblotting using a mouse anti-V5 primary antibody (Invitrogen, Carlsbad, CA) and HRP-conjugated goat anti-mouse secondary antibody (Pierce, Rockford, IL). Anti-V5 staining was detected by chemiluminescence, using the ECLTM westernblotting analysis system (Amersham Pharmacia Biotech, Buckinghamshire, UK). No staining was observed in cells transfected with empty vector alone (not shown). The results in figure ? show that SEQ ID NO: 4 is predominantly detected in the supernatant, indicating that SEQ ID NO: 4 is secreted or cleaved from the cell surface in HEK293 cells.

